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ON THE CULTIVATION OF THE BACILLUS OF LEPROSY BY THE METHOD OF CLEGG.

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This communication is to record our success in confirming the work of Clegg[a] in cultivating the bacillus lepra on artificial media. Our work having progressed to the point where an acid-fast bacillus has been grown on artificial media four times from three cases of leprosy and one of these obtained in pure culture, we think it proper to publish our work to date, reserving the question of cultural peculiarities and animal experimentation for a complete paper to be published later.

We are all the more anxious to publish this preliminary communication as our experience has shown that to workers less experienced than Clegg in amœba cultures the technical difficulties are so great that we fear that this most important advance will not receive the prompt recognition it deserves. We are frank to state that after four months' work we were about to abandon our experiments when the first culture was found growing in a series of inoculated tubes which were examined as a matter of conscientiousness before being discarded.

No attempt will be made to give a thoroughgoing description of the technique which is fully covered in Clegg's articles. We will rather emphasize certain points which seem important from our own experience.

I. *The amœba.*—The first amœba which we isolated was from pond water and was found unsuitable because it was so sluggish that it would not migrate from the center to the periphery of a plate and so could not be brought into "pure culture" with a single symbiotic bacterium of our own choice. Three other amœbas were subsequently isolated from three guinea-pig intestines and were finally brought into symbiotic relation with several known bacteria.

II. *The symbiant.*—After numerous trials we confined our work to the three strains of guinea-pig amœba in pure culture with a stock cholera spirillum, which came originally from the Hygienic Laboratory, United States Public Health and Marine-Hospital Service. Each of us isolated an amœba independently for our work, but all used the same cholera culture as a symbiant.

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[a] The Philippine Journal of Science, Vol. IV, pp. 77, 403. Also a personal communication at the time of Clegg's visit to this station, January, 1910.

III. *Culture media.*—We used the two agars recommended by Clegg. We think it important that these media be cleared with an egg in the usual manner. The media will hereafter be called Clegg I and Clegg II, the latter being that without beef extract.

IV. *Incubation.*—The stock cultures of amœba with cholera were carried along at room temperature (22° C.), while all cultures to which leprous material had been added were put at 36° to 37.5° C.

V. *Moisture.*—A large amount of water of condensation should be present in the culture tubes, and in Honolulu, where a part of the work was done, owing to the excessive dryness of the climate at this season of the year, it was found necessary to use an incubator with an air-moistening device or to put rubber caps on the tubes.

VI. *Selection of cases for material to inoculate.*—All our cases from which we succeeded in cultivating the bacillus were untreated or had received only a few doses of Chaulmoogra Oil and strychnine. Of course we chose individuals who showed active skin lesions of a tubercular sort.

VII. *Selection of lesions and technique of inoculation.*—An active lesion which was not undergoing purulent softening and was not ulcerated was rubbed with alcohol. The lesion was then firmly compressed with the fingers by an assistant, and while bloodless was incised with a sterile knife. The incision was carried to a depth of about 5 millimeters and given a similar lateral extent. The clear or faintly blood-stained serum which filled the incision was transferred by a platinum loop to the surface of the culture medium. We recommend the inoculation of only two such loops full for each tube and that they be stroked and not smeared over the whole surface. The reason for this is that the fewer organisms used in the inoculation, within reason, the easier it is for the beginner to convince himself when true multiplication occurs. Much confusion follows when heavy inoculations are made and it may not be convincing for several generations of the culture, owing to the difficulty of deciding between bacilli carried over and those which are growing.

VIII. *Preparation of cultures for inoculation.*—We all noted that if a twenty-four to thirty-six hour old amœba-cholera culture was inoculated with leprous juice that during the subsequent twenty-four to forty-eight hours there was a rapid disappearance of the bacilli, so that after two days it might be difficult to find a single clump of typical bacilli in a smear from the area of the culture media inoculated. One of us (D. H. C.) was able to show that this disappearance of the bacilli was associated with an active phagocytosis of the lepra bacilli by the amœba. To prevent this loss of organisms we resorted to the following modification of Clegg's technique. The amœba-cholera cultures were kept at 37° C. for five days before the leprous material was added. This incubation caused the encystment of the amœba, and all our successful cultures were obtained in this way. We strongly recommend this method, which, although it is only a variant of Clegg's repeated daily inoculation method, has this advantage—that when working in the Tropics, where all bacteria and molds abound, the less one opens a culture tube the better.

IX. *Rate of growth.*—In our early work we expected to find multiplication within ten days, and doubtless threw away many tubes which would have shown growth if incubated longer. Our present rule is not to open a primary inoculation tube for at least three weeks. By that time the examination of a smear will show in which tube multiplication is proceeding, and when such a tube is found no difficulty will be experienced in propagating the culture indefinitely. (We refer here to the mixed culture of amœba-cholera-lepra.)

SUMMARY.

We have each succeeded, working independently, in establishing amoeba-cholera-lepra cultures from cases of leprosy (D. H. C. 1, W. R. B. 2, H. T. H. 1). One of us (D. H. C.) has obtained a strain in pure culture by the heating technique described by Clegg. The amoebacholeralepra cultures have been carried through from three to ten generations. The pure culture is now growing in its third generation.

DISCUSSION.

We feel that the fact that we have been able to cultivate an acid-fast bacillus closely resembling the lepra bacillus four times from 3 cases of leprosy by following Clegg's method, taken in conjunction with his work, makes it exceedingly improbable that we are dealing with an accidentally introduced extraneous saprophyte. Of course we must suspend judgment till we can complete our control work and animal experimentation to exclude the tubercle bacillus, but we consider it so important for the advancement of our knowledge of leprosy that this work be taken up by others that we will publish our work so far as it has gone.

CONCLUSION.

An acid-fast bacillus resembling the lepra bacillus as seen in the human tissues can be isolated and grown in pure culture by the method of Clegg from the active skin lesions of cases of leprosy.